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five streams on Vancouver Island, B.C.**

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A MORPHOLOGICAL AND ELECTROPHORETIC COMPARISON
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BARRIERS ON FIVE STREAMS ON VANCOUVER ISLAND, B. C.

by

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ABSTRACT

Parkinson, E., R., J. Behnke and W. Pollard. 1984. A morphological and electrophoretic comparison of rainbow trout (Salmo gairdneri) above and below barriers on five streams on Vancouver Island, B. C. Fisheries Management Report No. 83.

Trout from above barriers in five remote streams were identified as rainbow trout with, in most cases, a slight cutthroat influence. Trout from below barriers were mostly rainbow, except for the Escalante River where some cutthroat trout and two possible hybrids were found. Downstream specimens were probably juveniles of anadromous steelhead stocks. Although individuals from upstream and downstream populations could not be consistently separated morphologically, in three streams there were differences in the mean scale counts and the incidence of basibranchial teeth. Gonadal development of small (< 200 mm) fish was the most obvious character distinguishing resident headwater populations from the anadromous downstream populations. Downstream populations were indistinguishable from each other electrophoretically but upstream populations differed both from each other and from their respective downstream populations at at least one electrophoretic locus. The management implications of our findings are discussed.

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INTRODUCTION

Headwater areas of British Columbia coastal streams, above barriers impassable to anadromous species, are generally inhabited by either rainbow trout (Salmo gairdneri) or coastal cutthroat trout (Salmo clarki clarki). Populations which are not associated with lakes usually consist of small, slow growing individuals which often cannot be clearly classified as either rainbow or cutthroat trout. Rainbow trout above barriers also may be true residents or juveniles of anadromous steelhead. In many rivers, barriers are passable to steelhead but impassable to other anadromous salmonids. It is important to know which form of rainbow is present above falls so that the appropriate set of regulations can be applied. This problem does not arise in the case of cutthroat trout where the anadromous form is rarely found above barriers impassable to other anadromous salmonids. In this study, the taxonomic status of trout in the headwater areas (above barriers) of five streams was determined morphologically and electrophoretically. Where rainbow were found, a second objective was to establish methods of differentiating between resident and anadromous forms.

Most headwater populations are currently unexploited due to inaccessibility to anglers and a small size which rarely exceeds the minimum legal limit for trout in streams. Non-anadromous headwater populations have historically been managed by the B. C. Fisheries Branch using regulations passed under the Federal Fisheries Act, but recent litigation has established that streams with fish that are not part of sport or commercial fisheries are outside the powers of the Federal Government to regulate (*Regina vs. MacMillan Bloedel Ltd.*, B.C. Court of Appeals, Application by Crown for leave to appeal denied by the Supreme Court of Canada). Regardless of their angling value, isolated populations may have educational, aesthetic and scientific values which are worthy of consideration. It is these additional values, which depend to a large extent on the uniqueness of the population, which we also wish to consider in this paper.

Uniqueness in headwater populations is dependent on the degree of isolation from other populations and the resulting development of unusual morphological, behavioral or physiological characteristics which may be worth preserving. In the United States, the importance of preserving endangered species and distinct population segments has been recognized through the passage of the Endangered Species Act of 1973 (Utter 1981). In this study, morphological and electrophoretic variation between the headwater populations, as well as between headwater and downstream populations, was used to confirm the isolation of some of the headwater populations, to provide evidence of the genetic divergence of the headwater populations and to provide a baseline sample for future studies.

The Study Area

Four of the streams flow directly into the ocean on the west coast of Vancouver Island but the Heber is a tributary of the Gold River (Fig. 1). Two headwater areas were sampled in the Heber, Saunders Creek and the Upper Heber. Some physical characteristics of each stream are given in Table 1. No water chemistry data is available but nutrient concentrations in other streams on the west coast of Vancouver Island are low, with TDS ranging between 15 and 50 mg/l. All four streams contain anadromous populations of various Oncorhynchus and Salmo species downstream of the obstructions. Dolly Varden (Salvelinus malma) were found upstream of the obstruction on Walbran Creek and may be in other headwater areas, as well. Only the Heber River was accessible by road and the possibility of contamination by transplanted fish is considered remote.

Table 1. Characteristics of the study streams and their obstructions.

	<u>Camper</u>	<u>Carmanah</u>	<u>Escalante</u>	<u>Heber</u>	<u>Walbran</u>
WATERSHED					
Area (km ²)	35	69.9	80	175	125
STREAM					
Length (km)	17	22	32	31	29
Gradient (%)					
Below Barrier	0-4	0-3	0-4	1-5	0-3
Above Barrier	1-5%	1-3	1	1-3	0-2
OBSTRUCTION					
Height (m)*	10	6-7	5	5.5	5
Elevation (m)	60	75	30 150	250	180
River Kilometer	2.5	2.5	7	8	13.6
Passability*	No	No	?	No	?

*Visual estimates

METHODS

Fish (70-170 mm) were collected by electroshocking and trapping above and below obstructions during the fall of 1979 and 1980. Fish were frozen within 12 hours of death and shipped to Vancouver, B.C. where liver and muscle samples were removed for electrophoresis. After preservation in formalin, they were then mailed to Fort Collins, Colorado for meristic analysis. In 1979, the samples were improperly frozen and electrophoretic data and pyloric caeca counts were not obtained. No electrophoretic



Fig. 1. Map of the study area.

data was obtained from the Heber River because this stream was not sampled in 1980.

Electrophoretic analyses were conducted using methods and equipment described by Parkinson (1980). Methods of separating cutthroat and rainbow electrophoretically are described by Campton and Utter (1985). The significance of observed differences between populations was tested with the G-test (Sokal and Rohlf 1981).

The basis of the morphological identification are previous characterizations of typical coastal rainbow trout, Salmo gairdneri irideus, and typical coastal cutthroat trout, Salmo clarki clarki. (Behnke 1979; Gold 1977; Schreck and Behnke 1971) Rainbow trout (S. g. irideus) are characterized by low scale counts (averaging about 120 to 135 in the lateral series and 24 to 30 scales above the lateral line), high pyloric caecal counts (typically 50 to 60), and a complete absence of basibranchial teeth (Behnke 1979). Coastal cutthroat trout (S. c. clarki) will average about 140 to 170 lateral series scales, 34 to 40 scales above the lateral line, about 40 pyloric caeca, and 100% occurrence of basibranchial teeth in pure populations. Other characters useful to separate coastal cutthroat trout from coastal rainbow trout are the longer head and jaw in cutthroat trout, narrower, elliptically shaped parr marks in cutthroat (vs. more rounded in rainbow), more heavily spotted adipose fin (often with black bordering edge) in cutthroat trout, and 9 pelvic fin rays in cutthroat trout vs. 10 pelvic rays in rainbow trout. There are fewer vertebrae in cutthroat (typically 60 to 63 vs 62 to 65). Rainbow trout generally have more gillrakers (18-21 vs 15-19) and better developed gillrakers (longer, attenuated vs. short, blunt) than coastal cutthroat.

RESULTS

Character Evaluation

The character evaluation of the 261 specimens from 11 sites of 6 western Vancouver Island streams reveals that 11 specimens from the lower Escalante River are the only cutthroat trout found in the collections. There are 40 specimens of rainbow trout and two specimens identified as hybrids from the lower Escalante samples. The rainbows are probably young steelhead and the cutthroat probably represent young of an anadromous population. The other samples are identified as rainbow trout, although most of them have indication of slight hybrid influence from cutthroat trout (based on characters such as basibranchial teeth, scales and gillrakers). Upstream and downstream samples are compared with each other and the standards for the two species in Fig. 2. There is an indication that Vancouver Island rainbow trout (and steelhead) average fewer pyloric caeca (40-50 vs. 50-60) than found in more southern populations of coastal rainbow trout. The samples collected in

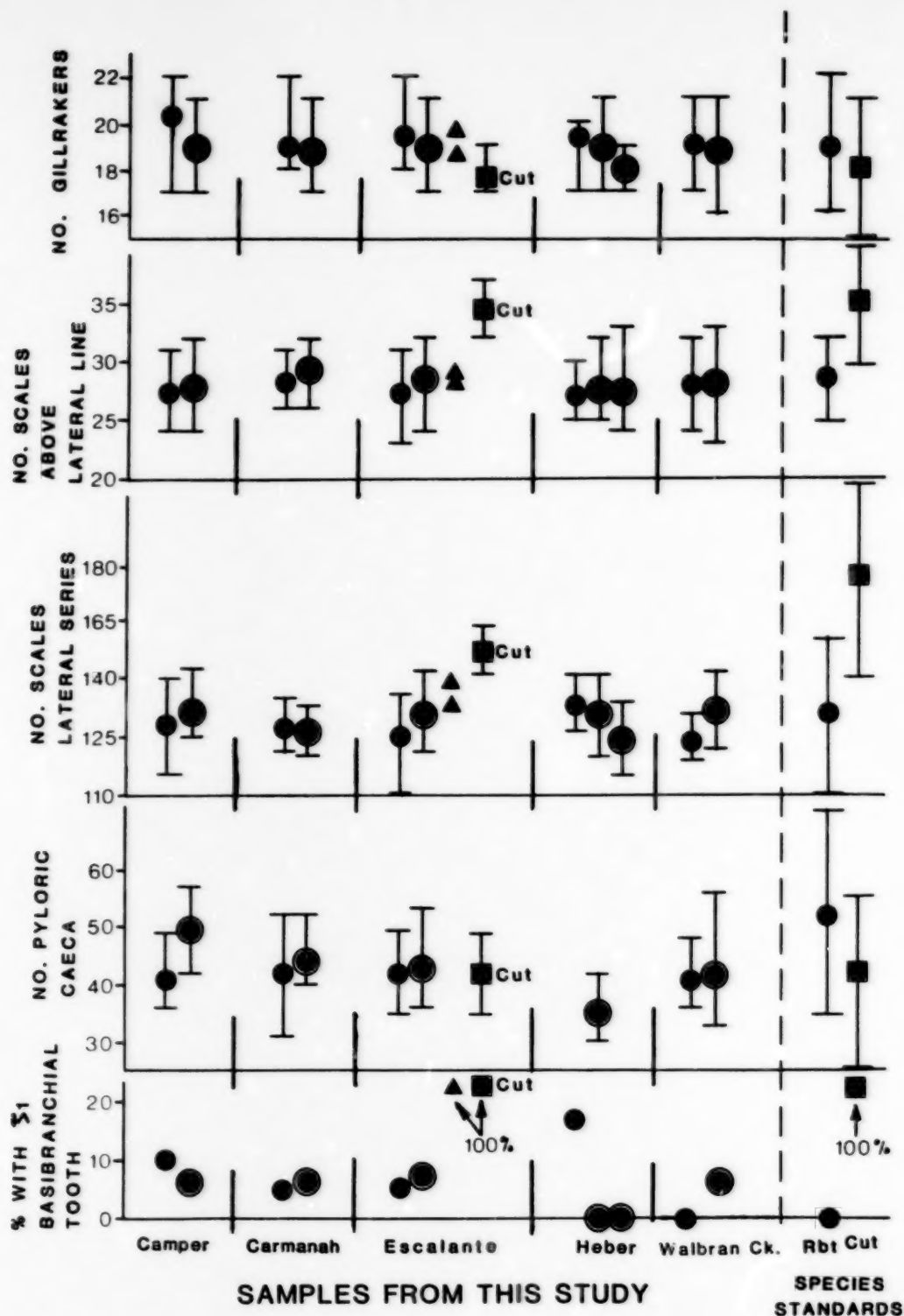


Fig. 2. Morphological characteristics of the upstream (○) and downstream (●) rainbow populations examined in this study. The cutthroat (■) from the Escalante are from the downstream area. Species standards from Behnke (1979), Schreck and Behnke (1971) and Gold (1977) are included for comparison. Bars indicate the range of values in each sample. Presumptive hybrids in the Escalante River are indicated by triangles (▲).

1979 were partially decomposed and character values are only "best estimates". The 1979 sample values do, however, closely agree with the better preserved 1980 samples when compared from the same collection site.

Nine specimens collected from Upper Saunders Creek in 1979 show no definite indication of a cutthroat trout hybrid influence. The gillraker count (17-19) is somewhat lower than expected for pure rainbow trout but the scale counts (average 27.5 above lateral line and 124 in the lateral series) are typical values for coastal rainbow trout. No specimen has any sign of basibranchial teeth.

None of the 15 specimens from the Upper Heber River in 1979 have basibranchial teeth, but scale counts (120-141 [131]) and more varied phenotypic appearance indicate a slight hybrid influence.

In contrast to other collections, the sample from the lower Heber River contains a more detectable cutthroat trout influence. Two of 12 specimens have a basibranchial tooth and scale counts average slightly higher (133) than in upstream samples.

The 27 specimens collected from the Upper Escalante River in 1979 are similar to the Lower Heber River samples. Two specimens have a basibranchial tooth and scale counts average 131.

Of 27 Lower Escalante River specimens collected in 1979, 26 are rainbow trout and one is a cutthroat trout. The cutthroat specimen clearly is distinguished by 19 basibranchial teeth (none in any rainbow specimen), 147 lateral series scales and 35 scales above the lateral line (vs. 109-134 [123.5] and 23-31 [27.2]) and 9 pelvic rays (vs. 10). Of 26 specimens collected in 1980, 10 are cutthroat trout. These specimens have 5 to 38 basibranchial teeth (18.8). This mean value is higher than has been found in any other sample of coastal cutthroat trout except for the original population found in Lake Sutherland, Washington. The scale counts of the cutthroat are clearly distinguished from the scale counts of 14 rainbow trout specimens with no overlap of counts. Differences are apparent in gillraker number and structure (17-19 [17.8] vs. 19-22 [19.6]). Both species from the lower Escalante River average 42 pyloric caeca. Two specimens of rainbow trout each have a single basibranchial tooth. These specimens have 19 and 20 gillrakers and 133 and 139 lateral series scales and are considered as hybrids, although it is unlikely that they are F1 hybrids. This was not confirmed by the electrophoretic analysis since these fish had rainbow phenotypes at all three diagnostic loci.

In the 1980 collection, 10 specimens from the lower Escalante were identified as cutthroat trout. Three enzyme systems (IDH, ME, SDH) were used to identify cutthroat trout

electrophoretically. Eight specimens had a cutthroat phenotype at all three loci and two had the cutthroat phenotype at only 2 (IDH, ME) of 3 loci.

The 54 specimens examined from upper Walbran Cr. are identified as rainbow trout with a slight cutthroat trout influence in their heredity, similar to the collections from the upper Escalante River and the Heber River. Two of 32 specimens collected in 1979 and 1 of 20 specimens of 1980 have basibranchial teeth. The phenotypic appearance suggests a cutthroat influence.

The sample of 15 specimens from lower Walbran Creek represent an unhybridized population of rainbow trout--probably juvenile steelhead. None have basibranchial teeth, the lateral series scale count averages 7-8 less than samples from above the falls and the gillrakers are better developed.

The upper Camper Cr. sample of 21 specimens collected in 1980 is typical of other slightly hybridized samples. Two of 21 specimens have a basibranchial tooth.

Samples of 20 specimens from the upper section and 17 specimens from the lower section of Carmanah Cr. appear virtually identical. Most of the specimens of more than 100 mm from above the falls, both males and females, have developing gonads indicating spawning the following spring (resident population). Two specimens from below the falls (123 and 128 mm) have developing testes. The gonads are undeveloped in the remaining specimens from below the falls, typical of young steelhead. Perhaps both resident and anadromous populations exist below the falls but the resident population cannot be differentiated from young steelhead except by degree of sexual maturation.

Electrophoretic Analysis

Nineteen loci were examined but only four (LDH-4, SOD, MDH-3, IDH-3,4) were found to be consistently polymorphic. The electrophoretic data on four streams indicated that downstream populations were typical of coastal steelhead populations but upstream populations diverged significantly at almost all loci. When compared to an average of previously sampled west coast of Vancouver Island steelhead populations (Parkinson 1980), none of the downstream populations differed significantly at any of the three polymorphic loci whereas 11 out of 12 of the comparisons involving upstream populations were significant (Table 2). In addition, heterogeneity at all three loci was absent among the downstream populations but present among the upstream populations. LDH comparisons are not included in Table 2 because of an almost total absence of variants in the upstream populations.

Unusually low frequencies of the variant allele were present in all four streams at the SOD locus and 3 out of 4

Table 2. Significance of comparisons between the sampled populations and an average of previously examined British Columbia steelhead populations from Parkinson (1980). Also included is the significance of the heterogeneity components between upstream and between downstream populations.

UPSTREAM	SOD	MDH	IDH
Camper	**	**	N.S.
Carmanah	**	**	*
Escalante	**	**	**
Walbran	*	*	*
Heterogeneity	**	**	**
DOWNSTREAM			
Camper	N.S.	N.S.	N.S.
Carmanah	N.S.	N.S.	N.S.
Escalante	N.S.	N.S.	N.S.
Walbran	N.S.	N.S.	N.S.
Heterogeneity	N.S.	N.S.	N.S.

*p < .05
**p < .01

Table 3. Gene frequencies and sample sizes at four loci in upstream, downstream and average west coast of Vancouver Island populations from Parkinson (1980). Numbers below enzyme names refer to the mobility of the allele relative to that of the most common allele (designated 100). LDH was not included in the significance testing on Table 3 because it was only slightly polymorphic. N refers to the number of individuals sampled. Only the frequencies of the most common alleles are given for SOD, MDH and LDH.

	N	SOD	MDH	IDH	LDH
Allele		100	100	100 72 49 124	100
UPSTREAM					
Camper	22	.95	1.00	.68 .17 .13 .02	1.00
Carmanah	20	1.00	1.00	.73 .27	1.00
Escalante	35	1.00	.57	.35 .03 .12 .50	1.00
Walbran	20	.82	.97	.54 .29 .17	.95
AVERAGE WEST COAST		.68	.85	.67 .18 .12 .03	.96
DOWNSTREAM					
Camper	19	.81	.89	.70 .20 .05 .05	.97
Carmanah	17	.73	.88	.71 .15 .14	.94
Escalante	22	.75	.88	.77 .18 .05	.95
Walbran	15	.60	.87	.69 .23 .07	.90

streams at the MDH loci (Table 3). At the MDH locus the remaining stream had a high frequency of the normally rare variant and at the IDH locus unusually high frequencies of variant alleles accounted for the differences which were significant. At the LDH locus, where there were too few variants to perform the G-tests, the variant was completely absent in 3 out of 4 upstream populations. Variants at all four loci were present at normal frequencies in all four downstream populations.

DISCUSSION

Only two Salmo subspecies, the coastal cutthroat trout (Salmo clarki clarki) and the coastal rainbow trout (Salmo gairdneri irideus) are native to the study area. Although a range of forms are now present in both subspecies (Nilsson and Northcote 1981, Cooper 1970, Neave 1949), we assume that each originated from their respective anadromous forms following the last glacial retreat. In sympatry, both species maintain their integrity and show some degree of habitat segregation as anadromous (Hartman and Gill 1968) and lacustrine (Nilsson and Northcote 1981) populations. Although few taxonomic studies are available, sympatric populations appear to be rare in small headwater streams. Both rainbow and cutthroat are found in the headwater tributaries of the Willamette River in Oregon but in this case the rainbow occupy the mainstem of the tributary and cutthroat are only found in the very smallest headwater streams (Nicholas 1978).

The headwater populations in this study were identified both morphologically and electrophoretically as rainbow but some morphological characters indicate a small cutthroat influence. Chromosome numbers, which differ markedly between the two species (Gold 1977) are not available for these populations. Reports of natural hybrids elsewhere (Campton and Utter 1985), the viability of hybrid offspring in hatcheries and the apparent absence of sympatric populations in headwater areas all indicate that cutthroat genes can be incorporated into a predominantly rainbow genome and vice versa. These and other subspecies of both rainbow and cutthroat readily hybridize and lose their identity (Behnke 1979). Hybridization between cutthroat and steelhead is, however, extremely rare in streams not stocked with hatchery fish (Behnke 1979). The most probable reason for this is the great difference in marine life history between the two species which would put hybrids with an intermediate type of life history at a severe competitive disadvantage relative to the parental species.

If basibranchial teeth are found in coastal rainbow trout, we assume that the hereditary basis for these teeth is the result of cutthroat trout genes in the population. None of the 15 specimens from lower Walbran Creek have basibranchial teeth, but one of 18 specimens from lower Camper Creek, and one of 17 specimens from lower Carmanah Creek have basibranchial teeth. We

assume this is a vestige of postglacial hybridization between steelhead and coastal cutthroat in this area. Over thousands of years, the genotypes have probably sorted out virtually all cutthroat influences and the present population can be considered as steelhead trout. An alternative hypothesis is that a resident population, consisting of slightly hybridized fish, maintains itself in isolation from steelhead trout below the falls in these streams. However, the specimens with basibranchial teeth from lower Camper and lower Carmanah creeks do not separate from other specimens in any other character. The two specimens with basibranchial teeth from lower Escalante Creek, however, do possess a different phenotypic appearance and relatively higher scale counts than the 50 specimens of rainbow trout collected from lower Escalante Creek. That is, these two specimens do not appear to be drawn from the same panmictic population of rainbow trout that is represented by the other 50 specimens. The Escalante River is a tributary to Nootka Sound, a bay and estuary system providing a suitable marine environment for sea-run coastal cutthroat trout (as evidenced by 11 specimens of Salmo clarki clarki).

It is therefore likely that, in contrast to the ancient hybridization responsible for the basibranchial teeth in rainbow trout south of Barkley Sound, occasional hybridization is a rare but an ongoing process in the Escalante River. The hybrids are probably strongly selected against because gene flow appears to be virtually nil between the samples of cutthroat and rainbow trout from the lower Escalante.

The presence of rainbow in the headwater areas of all five streams we sampled was unexpected. Cutthroat trout instead of rainbow are found in headwater areas of a number of streams on the east coast of Vancouver Island (Glova and Mason 1977, Tripp and McCart 1983), the lower Fraser Valley area of mainland British Columbia (Hartman and Gill 1968) and the Olympic Peninsula (Michael 1983). Barkley sound, at roughly the mid point of our sampling area, has at least three streams with headwater cutthroat trout populations (Andersen 1983 and G. Hartman, pers. comm.). An inventory of streams in the Brooks Bay area north of our sampling area found cutthroat in the headwaters of Buck Creek (P. G. Bruce, pers. comm.).

Gonadal development was the only character that could be used by field staff to differentiate resident and anadromous populations. Gonadal development in males was not as useful a character because precocious males, which mature before migration to the sea occur in a number of anadromous species, including steelhead. Rainbow trout spawn in the spring but the gonads of maturing fish are already enlarged the previous autumn. Samples for gonad development can therefore be taken in either autumn or early spring. In this study the occurrence of sexually maturing fish from above and below falls was determined only in one stream but the largest headwater fish captured in the five streams was 162 mm, with the maximum size generally between 140 and 150 mm.

A high percentage of maturing fish between 100-200 mm would confirm the presence of resident stocks.

Other characters that could be used, such as coloration and spotting pattern, are subjective. Experienced specialists familiar with subtle differences such as gillraker shape, who could evaluate a variety of characters to get a composite view of the population, may be able to separate stocks but it is questionable that many field staff could do this consistently. We did not examine otolith nuclei which have already been used to differentiate anadromous and resident forms (Ryback et al 1975). This method depends on differences in egg size and should work for these populations.

Differences in electrophoretic gene frequencies leave little doubt that each of the upstream populations is reproductively isolated from their downstream counterparts, as well as each other. This isolation has probably been present since the isostatic rise of the glacially depressed land, approximately 10,000 years ago (Matthews et al. 1970), caused the sea level to drop below the level of the falls. If an obstruction is a complete barrier to upstream movement, then downstream populations cannot contribute to the gene pool of upstream populations. In upstream populations, the strong selection against downstream movement should result in a reduction in the tendency to move downstream (Northcote 1981) and upstream resident populations apparently do not contribute to downstream anadromous populations (Michael 1983). Electrophoretic differences between upstream and downstream populations support the assertion that isolation between upstream and downstream populations is essentially complete in the study streams.

Our observations indicate that some genetic differentiation has taken place. Electrophoretically, there have been gene frequency changes at polymorphic loci with possible fixation of the most common allele in a number of cases. No new or even unusual alleles were observed at any of the loci that were examined. Morphologically, little change has taken place in meristic characteristics and these populations are all still recognizable as rainbow trout. Differences in color patterns and brightness of color which are difficult to quantify, but which are obvious to an experienced observer, are the main differences in external appearance. There are, of course, many other traits which were not examined where differences between these populations may be evident, an example being differences in migratory behavior documented by Northcote (1981).

Despite their isolation these populations have not differentiated from each other to the same extent as have the more obviously distinct southern subspecies of cutthroat and rainbow described by Behnke (1979), Gold (1977) and Schreck and Behnke (1971). Most of the populations described by these authors were isolated prior to the Wisconsin glaciation and can

legitimately be called subspecies, whereas the Vancouver Island populations are fairly recent invaders which we assume were originally part of the coastal rainbow group until 5,000 to 10,000 yrs B.P. From a fisheries management point of view the recent isolation, the lack of differentiation, the large number of headwater populations, and the small size at maturity makes the preservation of all but a small number of populations relatively unimportant. From a scientific point of view, however, the possibility of studying the pattern of differentiation in a large number of populations which were isolated at approximately the same time argues for the preservation of as many of these populations as possible.

The main threats to resident headwater populations of trout are habitat destruction, angling and the introduction of non-native fish. Habitat destruction, principally from logging, can result in large declines in trout populations but various protective measures appear to ameliorate much of this effect (Moring and Lantz 1975) and extinction is unlikely except in the most extreme cases. Angling is not considered to be a serious threat at present because these populations are not particularly attractive to anglers due to their inaccessibility and small size at maturity. Streams near population centers may be an exception to this generalization because of heavy use by juvenile anglers catching fish that are mostly of sub-legal size under current regulations. As sport fishing pressures increase, it will be necessary to identify those areas with sport fishing potential and design regulations necessary to maintain populations at acceptable levels. In summary, both habitat destruction and angling may cause large declines in population size but the probability of genetic change is small unless the decline is so great that genetic drift becomes a factor.

In contrast, the introduction of foreign races of either rainbow or cutthroat trout into headwater areas may result in genetic changes in resident populations if foreign genes are incorporated into resident gene pools through hybridization. Although non-authorized introductions do take place, introductions by government agencies are the most likely source of contamination. Rainbow and cutthroat trout have been intentionally introduced into many headwater lakes, and therefore their associated streams, since the 1930's but introductions into stream only systems were rare. More recently, a program of steelhead fry introductions in headwater areas has been initiated in order to utilize these areas to rear smolts to augment downstream populations. Although most of these fish will emigrate, precocious male parr, which mature before emigration, are present in many Atlantic salmon populations (Dalley et al. 1983; Myers 1984) and steelhead populations (Hartman and Gill 1968; Schmidt and House 1979).

Protection of the genetic integrity of headwater trout populations and protection of the production capacity of streams are to some extent separate issues. Threats in the latter case

include overfishing and habitat destruction whereas the introduction of foreign trout, which may enhance production, is the most serious threat to the genetic integrity of headwater stocks. Protection of the productive capacity of headwater streams which contain exploitable fish populations (including areas such as spawning and rearing tributaries of lakes) is justifiable regardless of their genetic uniqueness. We have shown that currently unexploitable populations to which no introductions have been made, are to some extent genetically unique and thus have a certain scientific and educational value which justifies their protection. Preservation of all headwater populations may be impractical but a coordinated effort should be made to preserve both the habitat and genetic integrity of a representative sample of these populations.

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